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Preliminary Studies on the Microbial Degradation of Plastic Waste Using *Aspergillus niger* and *Pseudomonas* sp.

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Abstract

The possibility of microbial degradation of plastic waste was investigated by isolating microorganisms present in dumpsite containing low-density polyethylene (LDP). *Aspergillus niger* (fungi) and *Pseudomonas* sp. (bacteria) were identified and subsequently used to biodegrade plastic waste. The medium was made up of 0.2 g of $MgSO_4$, 1.0 g of KH_2PO_4, 1.0 g of K_2HPO_4, 1.0 g of NH_4NO_3, 0.02 g of $CaCl_2$, 0.05 g of $FeCl_3$ in 1000 ml water. 10 ml of the medium containing the bacteria and/or fungi was poured into test tubes and 0.1 g of the plastic sample (Pure water sachet) pre-treated with ethanol was introduced into the tubes. The pH of the medium was adjusted to 7.2, 5.4 and 6.0 for *Pseudomonas* sp., *Aspergillus niger* and the mixed culture respectively. Each experiment was carried out aerobically at room temperature and incubated on a rotary shaker at 120 rpm. The weight loss in each experiment was monitored at 10 days interval for 60 days. The total weight loss after 60 days was 7.2%, 12.4%, 15% for degradation with *Pseudomonas* sp., *Aspergillus niger* and the mixed culture respectively. From this study it can be inferred that *Pseudomonas* sp. and *Aspergillus niger* have the ability to degrade plastics. It can also be inferred that *Aspergillus niger* degraded plastics better than *Pseudomonas* sp. and there was synergy between the two microorganisms since the mixed culture gave a higher degradation.

Keywords

Biodegradation, Synthetic Plastic, Low Density Polyethylene, *Pseudomonas* sp., *Aspergillus niger*, Biodegradability

1. Introduction

Plastic is a synthetic polymer. It consists of carbon, hydrogen, silicon, oxygen,

chloride and nitrogen. It is derived from different sources such as oil, coal and natural gas. Plastics are extensively used because of their stability and durability. There are different types of plastics, and examples are polyethylene (PE), Poly Ethylene Terephthalate (PET), Nylons, Poly-Propylene (PP), Polystyrene (PS), Polyvinyl Chloride (PVC), and Polyurethane (PUR) [1]. Due to the absence of efficient methods for safe disposal of these synthetic polymers, they often end up accumulating in the environment, thus posing an ever-increasing ecological threat [2]. The environmental concerns include air, water and soil pollution.

Plastic can be degraded by a variety of mechanisms such as chemical, thermal, photooxidation and biodegradation, all of which take an extremely long time depending on the molecular weight of polymer, it could take up to 1000 years to degrade some types of plastics [3].

Microorganisms can play a vital role in this process, as over 90 genera of bacteria, fungi and actinomycetes have the ability to degrade plastic [4] [5] [6] [7]. Generally, the biodegradation of plastic by microorganisms is a very slow process, and some microorganisms cannot degrade certain plastics [8]. Different types of microbes degrade different groups of plastics, for example, *Pseudomonas* sp*. and Bacillus cereus* obtained from a Plastic dumpsite degraded polythene with degradation efficiency of 12.5% [9] and *Aspergillus glaucus and Pseudomonas* sp. obtained from mangrove soil degraded polythene and plastic with degradation efficiency of 20.8%, 7.26%, 20.54% and 8.16% respectively [10].

Also, in other studies, *Aspergillus niger and Streptococcus lactis* obtained from sewage water soil, sludge area soil, agricultural soil were used to degrade polythene bags and plastic cups and gave degradation efficiency ranging from 12.25% - 12.5% [11]. *Bacillus cereus* obtained from a dumpsite has also been used to degrade low-density polyethylene that gave degradation efficiency of 2.4% - 7.4% [12]. *Streptomyces sp.* obtained from garbage soil has degraded 46.7% of low-density polyethylene [4], while 75.3% degradation of plastic milk cover have been reported [6] using *Pseudomonas putida* obtained from garden soil. There was also a report on the use of *Micrococcus luteus* obtained from forest soil to degrade plastic cups that gave a degradation efficiency of 38% [7].

These previous studies established the fact that microorganisms in their pure culture have the capacities to degrade plastics but have not considered the ability of mixed culture of microorganisms. Therefore, this work aims at studying the abilities in both pure and mixed cultures of isolated *Pseudomonas* sp. and *Aspergillus niger* obtained from a dumpsite in the University of Lagos, Nigeria to degrade plastic.

2. Materials and Methods

2.1. Inoculum Preparation

Samples of soil from a dump site in the University of Lagos which has had contact with plastics of different forms for a long period of time was collected in a sterile sample bottle from the depth of 5 - 10 cm. From the soil samples, indi-

genous microorganisms were isolated using convectional serial dilution and selective agar methods. Nutrient agar and potato dextrose agar were used to selectively grow bacteria and fungi respectively. The most prevalent microbes were subjected to further identification using staining and diagnostic morphological feature of genera through macroscopic and microscopic examination.

The loops of selected strains, *Pseudomonas* sp. *and Aspergillus niger* for this study were transferred into prepared basal mineral salt medium to be used as inoculum for the study.

2.2. Nutrient Basal Media Contents

The Bushnell and Haas agar [13] was used for testing the ability of microorganisms in degrading plastics. The media was prepared by adding 0.2 g of $MgSO_4$, 1.0 g of KH_2PO_4, 1.0 g of K_2HPO_4, 1.0 g of NH_4NO_3, 0.02 g of $CaCl_2$, 0.05 g of $FeCl_3$ into 1000 ml of tap water. The pH of the medium was adjusted to 7.2 (Bacteria only), 5.4 (Fungi only), 6.0 (Bacteria and Fungi) and the medium autoclaved at 121˚C for 15 minutes.

2.3. Experimental

The ability of isolated bacteria and fungi in pure and mixed cultures to degrade the Low Density Polyethylene (LDP) (popularly called Pure water sachet in Nigeria) was carried out using sacrificial test-tubes method containing 10 ml of the basal mineral salt medium, 600 ul of the inoculum and a strip of the LPD weighing 0.1 g, which has been washed with 70% ethanol thoroughly, then rinsed with distilled water aseptically. The initial concentration of bacterial and fungal inoculum was maintained at 0.5 McFarland Standard. The tubes were incubated on a rotary shaker (120 rpm) at room temperature of 25˚C. Sampling was carried out aseptically at 10, 20, 30, 40, 50 and 60 days after incubation and checked for weight losses. A set of control experiments containing only the pure water sachet sample in basal nutrient medium devoid of bacterial and/or fungal inoculum carried out.

2.4. Measurement of Residual Substrate

Weight Loss Measurements: The test tubes containing the plastic samples after exposure to the bacteria and/or fungi was taken and washed thoroughly with ethanol. The strips were then dried at 60˚C through the night and the percentage weight loss was determined using the following formula:

$$\text{Weight loss}(\%) = \frac{\text{initial weight} - \text{final weight}}{\text{initial weight}} \times 100 \quad (1)$$

2.5. Cell Growth (Biomass) and Kinetics

An aliquot from the incubated tubes was taken at regular intervals within the 60 days of incubation for quantification of bacterial and/or fungal cells. This was done using a UV Spectrophotometer at a wavelength of 600 nm to obtain the

absorbance which was then used to get the biomass growth. The controls were also tested regularly for contamination. The correlation between the absorbance and the biomass (microbial) growth is given as:

$$\mathrm{OD}_{600} \text{ of } 1.0 = 8\times10^{8} \text{ cells/ml}$$

$$X = \frac{8\times10^{8}}{1} \times \text{Optical Density} \tag{2}$$

While the growth kinetics of the experiments are using $In\frac{X}{X_o} = \mu\left(t - t_0\right)$

where X, t and μ are biomass concentration, time and specific growth rate respectively while subscript o means initial.

3. Results and Discussions

Biodegradation of Plastic Sample

The rate of biodegradation of LDP as evaluated using Equation (1) presented in Figures 1-3. Figure 1 showed the relationship between the substrate concentration and time. From the graphs plotted we can deduce that the substrate concentration decreased as time increased for the 3 experiments that were monitored.

Over the course of 60 days at a 10 days interval, the weight loss of the pure water sachet with an initial weight of 0.1 g in 10 ml (10 g/L) bacterial and/or fungal medium was monitored. The total weight loss after 60 days was 7.2%, 12.4%, 15% for the bacterial, fungal and mixture of both respectively. This compared to 12.5% degradation efficiency obtained using Pseudomonas sp obtained from plastic dumpsite to degrade polythene by [9]. Also, in the work of Priyanka and Archana, 12.25% and 12.5% degradation of polythene bags and plastic cups were obtained respectively using *Aspergillus niger* isolated from soil [11]. The percentage weight loss over 60 days at a 10 days interval is shown in Figure 2. From the results obtained it showed that the combination of both *Aspergillus niger* and *Pseudomonas* sp. gave the highest degrading efficiency. It can also be deduced that *Aspergillus niger* had a greater impact on the degradation as it gave a better percentage reduction compared to *Pseudomonas* sp. when the pure cultures of the microbes were used.

The biomass growth was monitored by using the optical density of the sample taken at 7 days interval for 28 days and hence the specific growth rate was obtained. Figure 3 showed plots of biomass growth against substrate concentration for each of the experiment. It can be seen from the plots that the substrate concentration decreased as biomass growth increased an indication that decrease in the weight of LDP was due to microbial degradation.

Biomass growth in the experiment where *Aspergillus niger* only was used had the highest biomass growth which showed the conditions favored its growth. This was followed by the experiment containing both *Aspergillus niger* and *Pseudomonas* sp. The mixed culture did not have the highest biomass growth

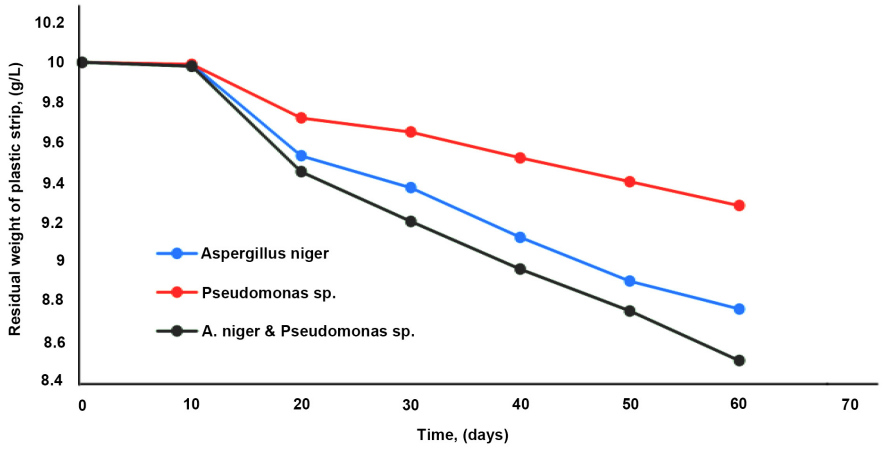

Figure 1. Plot of residual weight against time.

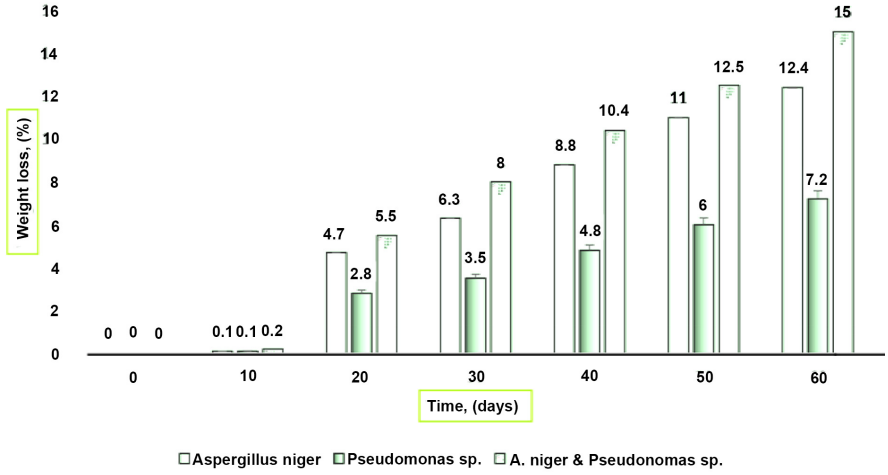

Figure 2. Graph of weight loss against time.

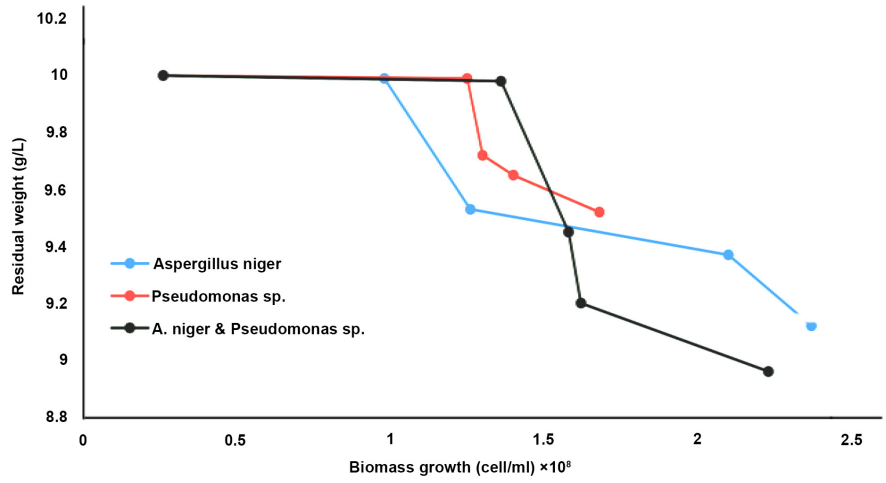

Figure 3. Graph of residual weight against biomass growth.

but the extent of degradation was higher, an indication that the mixed culture was stable than the monocultures.

4. Conclusions

Accumulation of plastic waste is a serious environmental issue. Biodegradation of plastics can be viewed as one of the strategic studies to overcome this problem. In this study, the biodegradation of plastic (pure water sachet) using *Aspergillus niger* (Fungi), *Pseudomonas* sp. (Bacteria) and the combination of both, isolated from soil samples gotten from the University of Lagos dumpsite were evaluated. The weight loss of each experiment monitored over a period of 60 days was 7.2%, 12.4% and 15% for *Pseudomonas* sp., *Aspergillus niger* and the combination of both respectively.

It can be inferred that *Pseudomonas* sp. and *Aspergillus niger* have the ability to degrade plastics. From the results obtained it can also be inferred that *Aspergillus niger* has the better ability to degrade plastics than *Pseudomonas* sp. under the conditions used for the experiment. The study also showed that the combination of both *Pseudomonas* sp. and *Aspergillus niger* gave the highest degrading efficiency for the experiments carried out.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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